Journal of Cranio-Maxillo-Facial Surgery 47 (2019) 695–703

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

A new multilayered membrane for tissue engineering of oral hard- and soft tissue by means of melt electrospinning writing and film casting – An in vitro study

Andreas Fuchs [a, *], Almoatazbellah Youssef [b], Axel Seher [a], Stefan Hartmann [a], Roman C. Brands [a], Urs D.A. Müller-Richter [a], Alexander C. Kübler [a], Christian Linz [a, c]

[a] Department of Oral and Maxillofacial Plastic Surgery, (Head: Prof. Dr. Dr. Alexander C. Kübler), University Hospital Würzburg, Pleicherwall 2, 97080 Würzburg, Germany
[b] Department for Functional Materials in Medicine and Dentistry, (Head: Prof. Dr. Jürgen Groll), University Hospital Würzburg, Pleicherwall 2, 97070 Würzburg, Germany
[c] Interdisciplinary Center for Clinical Research, University Hospital Würzburg, Josef-Schneider-Straße 2, 97070 Würzburg, Germany

ARTICLE INFO

Article history:
Paper received 17 October 2018
Accepted 29 January 2019
Available online 4 February 2019

Keywords:
Melt electrowriting
Polycaprolactone
Guided tissue regeneration
Scaffold
3D printing

ABSTRACT

Membranes that form a mechanical barrier not only for cells but also for the bacterial flora of the oral cavity may be helpful in infection-free wound healing for guided tissue regeneration (GTR) applications in the field of oral- and maxillofacial surgery. Controlled wound healing without interference from bacterial contamination appears to be achievable in combination with surface scaffolds for bone- and soft tissue regeneration. As this has not yet been realized, we developed multilayered membranes in this study consisting of specific surface scaffolds for bone- and mucosal regeneration as well as bacteria-tight core membranes. These membranes were evaluated in terms of cell growth of osteoblast- (MG63), keratinocyte- (HaCaT), and fibroblast (L929) cell lines. Scaffolds were fabricated via melt electrospinning writing (MEW), while the core membrane was produced via film casting. All constructs were made of medical-grade poly(ε-caprolactone) (PCL). The bacteria-tightness was tested via a bacterial transmigration-assay. PCL scaffolds and core membranes alone demonstrated good cytocompatibility for all cell lines, which was even enhanced by fusing both components together. The core membrane displayed complete bacteria-tightness over two weeks. These bacteria-tight, individually-designed membranes from medical-grade PCL represent a high-potential, clinically oriented method of GTR in the field of oral- and maxillofacial surgery.

1. Introduction

Membranes are currently used in oral and maxillofacial surgery mostly for bony defects. This use comprises inter alia - pre-prosthetic indications, pre-implant augmentations (e.g. sinus floor elevation), ridge augmentation and ridge preservation (Vignoletti et al., 2012; Schwarz et al., 2015; Jonker et al., 2016), coverage of bone trauma (especially in the midface and orbital floor) (Rinna et al., 2005; Becker et al., 2010; Birkenfeld et al., 2015), and hemostyptic indications (Yonezawa et al., 2012; Arai et al., 2012). These indications are mostly subject to the principle of GTR, or more specifically, to that of guided bone regeneration (GBR). In GBR, membranes form a barrier to protect slowly regenerating bone tissue from an interposition of faster-proliferating tissues, such as fibrous tissue or epithelium (Dimitriou et al., 2012). In the oral cavity, membranes are in direct contact with bone, fibrous tissue, and epithelial tissue, especially regarding pre-implant indications. The membranes used for these indications should therefore provide adequate growth conditions for specific tissues to allow for the proper healing of bone as well as the soft tissues that cover it (Behring et al., 2008; Scheller et al., 2009). Moreover, it would be beneficial if membranes served as a barrier for the omnipresent oral bacterial flora, which always poses a risk for wound infections, particularly for vulnerable structures, such as (augmented) bone (Zhang et al., 2010). This

* Corresponding author. Fax: +49 931 201 72725.
E-mail address: fuchs_a2@ukw.de (A. Fuchs).

https://doi.org/10.1016/j.jcms.2019.01.043
1010-5182/

benefit is of even greater importance in the treatment of immunocompromised patients, such as individuals who suffer from diabetes, irradiation in the head- and neck area, as well as patients on anti-resorptive medication (Jegoux et al., 2010; Mardas et al., 2011; Erdogan et al., 2015). Membrane systems that are applied clinically currently face certain disadvantages in terms of sufficiently preventing infections. One approach to preventing bacterial infections is to apply antibiotic substances to the membranes (Xue et al., 2015); however, in this study, we investigated the possibility of fabricating a membrane by means of film casting, which represents a mechanical barrier to prevent bacterial transmigration through the membrane. In a second step to provide an optimized substrate for cell growth of bone and epithelial cells upon these bacteria-tight membranes, surface scaffolds were fabricated via melt electrospinning writing (MEW). This is an innovative 3D printing technology that allows for the fabrication of individually planned high-precision structures out of polymer melts (Dalton et al., 2013; Brown et al., 2016). In MEW, polymers can be varied depending on what material properties are desired. Resorbable membranes are favourable in most oral- and maxillofacial applications. Resorbable polymers such as poly(lactide-coglycolide) (PLGA), polylactide (PLA), and poly(ε-caprolactone) (PCL), which was chosen in this study, can serve as basic material for the MEW process (Sampath et al., 2014; Qin et al., 2015). In this procedure, an electrically charged, viscous polymer jet is sustained at low flow rates from a nozzle using an applied voltage and "drawn" through the air in the direction of a collector with the opposite electrical potential, where the fibers form well-defined structures layer by layer with up to submicron diameters (Dalton et al., 2006; Hochleitner et al., 2015). MEW is distinct to the more common technique of solution electrospinning, where the voltage is applied to create electrical instabilities, and involves dissolving polymers in often toxic solvents with a risk of solvent residuals in the fibers. Furthermore, an exact deposition of polymer fibers in solution electrospinning is not possible due to excessive electrostatic forces within the fiber jet (Hutmacher and Dalton, 2011; Brown et al., 2014; Chen et al., 2016; Lian and Meng, 2017). In this study, we used MEW to fabricate individually designed surface scaffolds consisting of medical-grade PCL. For the surface scaffolds for the bone side, we chose a box-shaped design as similar designs have already proven suitable in the growth of osteoblast- and endosteal cells (Muerza-Cascante et al., 2017; Baldwin et al., 2017). The fabrication of electrospun scaffolds for tissue engineering of mucosa tissue has been mostly investigated thus far for solution electrospinning but not for MEW (Santocildes-Romero et al., 2017). Therefore, we experimented with scaffolds with triangular pores as MEW mucosal scaffolds. Core membranes were then fused with MEW scaffolds, resulting in individual bilayered membranes. Such individualized membranes - which combine bacteria-tightness with good conditions for the cell growth of oral tissues - represent an interesting new approach in tissue engineering. High-precision membranes that are tailored to the needs of different tissues and have the potential to prevent infections of underlying tissues thus represent a promising area of contribution to improved wound healing/tissue regeneration in oral and maxillofacial surgery.

2. Materials and methods

2.1. Materials

All scaffolds and membranes consisted of medical-grade PCL PURASORB® PC 12 (Corbion Inc., Gorinchem, Netherlands), which was divided into 50 mL falcon tubes in an argon atmosphere and then stored at −80 °C until used.

2.2. Fabrication via MEW

Scaffolds were produced by a custom-made MEW device (Fig. 1) as described elsewhere (Hochleitner et al., 2015). To melt the PCL inside the device, the polymer was heated to 73.0 °C ± 1.0 °C in a disposable plastic syringe with a 22G nozzle attached to it (Nordson EFD Deutschland GmbH, Pforzheim, Germany). An acceleration voltage of 6 kV was chosen between the tip of the nozzle and the stainless-steel collector and a pneumatic pressure of 1.2 bar was applied to the molten PCL. The nozzle-to-collector distance was 4 mm, and the collector moved at a speed of 400 mm/min. Scaffold fabrication was conducted at an average humidity level of 38.5 ± 3.5% and an ambient temperature of 21.4 ± 0.4 °C.

2.3. Scaffold designs

All scaffolds consisted of fibers with diameters of approximately 20 μm. Box-structure scaffolds (Fig. 2a) were fabricated via MEW for all experiments by alternating layer deposition via 0° layers and 90° layers (15 × 15 mm^2) in each direction with turning loops. Five layers were deposited in each direction (0° and 90°) such that 10 fibers overlapped at the intersections. In this manner, scaffolds with fiber diameters of 20 μm and fiber spacings of 250 μm and 500 μm were fabricated. Triangular-structure scaffolds (Fig. 2b) were fabricated via MEW in a similar manner. Layer deposition proceeded here via 0° layers, 60° layers and 120° layers (15 × 15 mm^2) in each direction. 8 layers were deposited in each direction (24 fibers overlapped at intersections). A fiber spacing of 250 μm was chosen. 100% ethanol was used to separate the scaffolds from the metal collector for both designs.

2.4. Film casting

For the film casting of the barrier membrane, a custom-made stainless-steel slide was filled with PCL granulate, which was heated up to approximately 73.0 °C with a heat gun until the granules were fused together and the melt showed a glazed surface (Fig. 3). At that point, the steel slide (including the PCL melt) was moved transversally over a degreased glass plate by a film applicator (COATMASTER 510, Erichsen, Hemer, Germany). One wall of the steel slide had a distance to the ground of 80 μm. Thus, a continuous PCL-film was deposited contrary to the advancing direction of the slide. A feed rate of 1 mm per second proved to be ideal for our purpose. The thickness of the produced film was measured by magnetic induction measurement and averaged 57 μm.

2.5. Fabrication of bilayered scaffold-core fusion products

The fusion of scaffolds and core membranes was performed via heat sealing. For this purpose, core membranes were heated to a temperature of approximately 70 °C on a degreased steel slide that was placed on a heating plate. When the core surface began to cool after becoming glazed, scaffolds were placed upon it and immediately chilled in cold deionized water.

2.6. Cell culture

Human osteoblast-like cell line MG63, keratinocyte cell line HaCaT, and fibroblast cell line L929 were cultivated in Dulbecco's modified Eagle's Medium (DMEM, Invitrogen, Karlsruhe, Germany), which was supplemented with 10% fetal calf serum (FCS, Invitrogen, Karlsruhe, Germany) and 1% penicillin and streptomycin (P/S, Invitrogen, Karlsruhe, Germany) in a pre-warmed (37 °C) and humidified atmosphere with 5% CO_2. The culture medium was changed every 3 days.

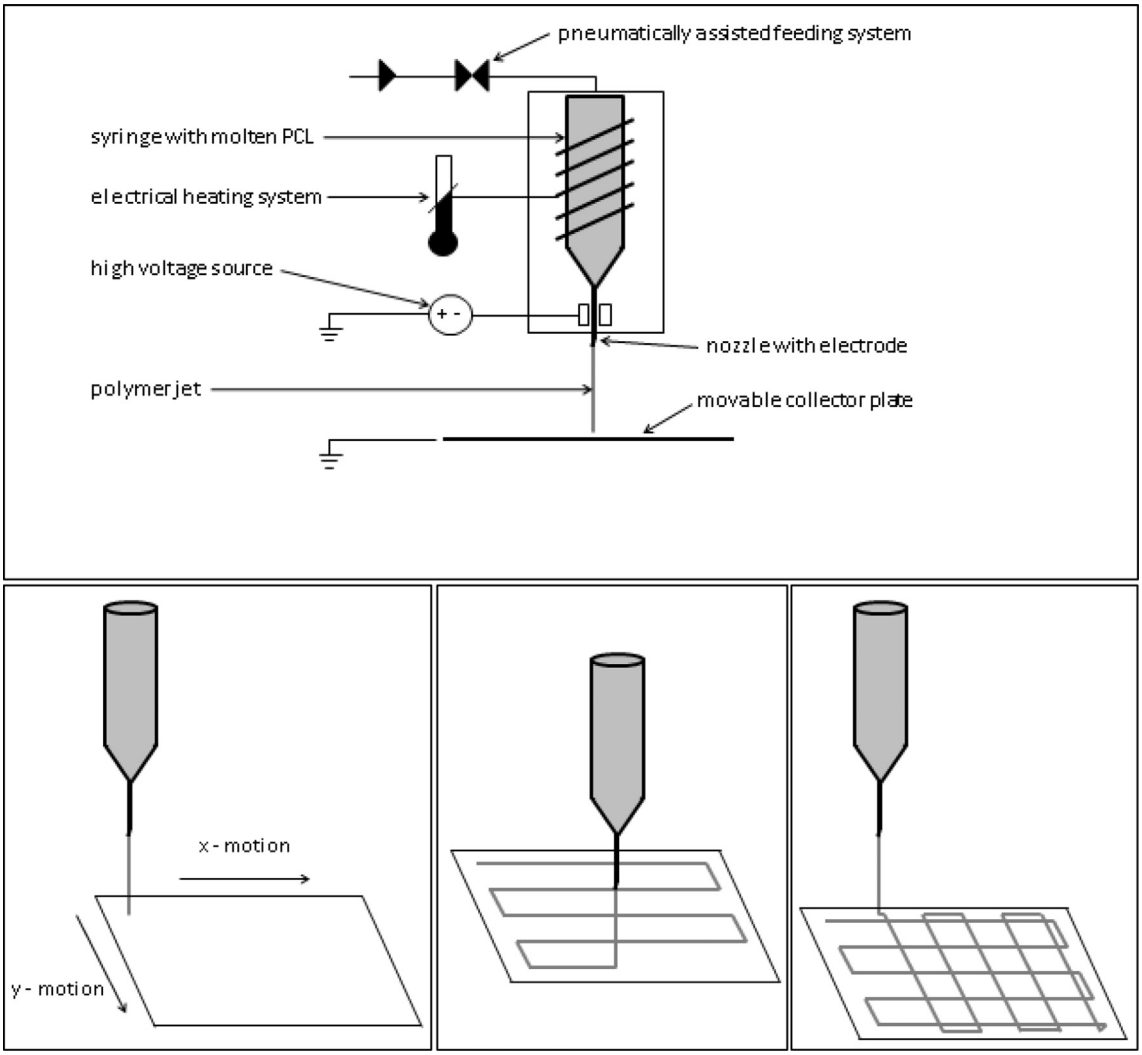

Fig. 1. Design of MEW device and schematic of the MEW process of the scaffolds.

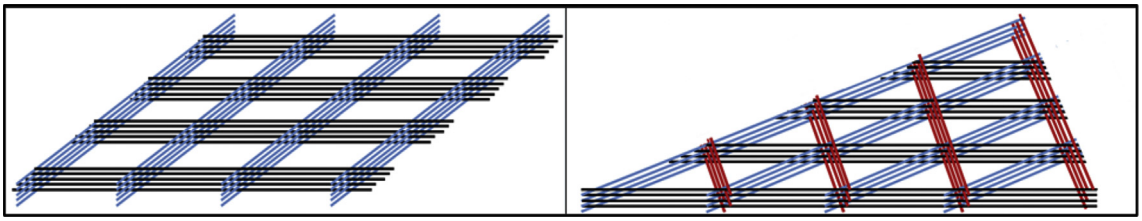

Fig. 2. Schematic of different scaffold designs. a) Box-structure scaffold, b) triangular-structure scaffold.

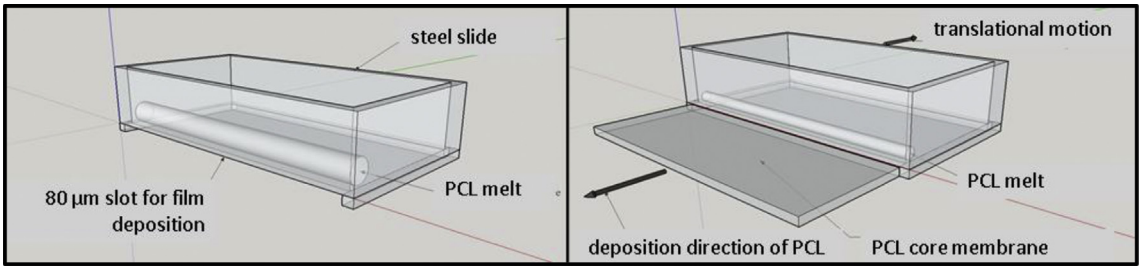

Fig. 3. Schematic of film casting process of core membranes.

In vitro experiments were performed in 12-well multi-well plates (Thermo Fisher Scientific, Waltham, USA). Scaffolds and membrane cores were placed into a cavity and weighed down by a glass ring and glass beads to keep them from floating in the cell culture medium. Positive control groups of cells with a glass ring as well as negative control groups consisting of pure medium or pure medium with a glass ring and beads were added.

2.6.1. MEW surface scaffolds

MG63 cells were seeded upon the box-structured scaffolds for bone regeneration in an initial concentration of 3.0×10^5 cells per scaffold to investigate the growth behaviour of different cell types on respective scaffolds over a period of 28 days. HaCaT and L929 cells were seeded in concentrations of 6.0×10^5 (HaCaT) and 3.0×10^5 (L929) cells on triangular-structured scaffolds for mucosal regeneration. Different seeding concentrations of respective cell lines were chosen due to their individual growth rates. The measurement of cell viability via WST-1 (Water-soluble Tetrazolium) assay was performed after 7, 14, and 28 days.

2.6.2. Core membrane

To assess the initial growth behavior on the PCL core membrane, MG63 and HaCaT cells were settled in concentrations of 1.0×10^5 cells and 3.0×10^5 cells per membrane, respectively. A viability measurement was performed via WST-1 assay on days 2, 3 and 4 after the initial settlement.

2.6.3. Bilayered membranes

Cell types MG63, HaCaT, and L929 were investigated. The measurement of cell viability was performed once on day 7. MG63 cells were seeded upon box-structured fusion products (bilayers) in an initial concentration of 3.0×10^5 cells per membrane, and HaCaT and L929 cells were seeded in concentrations of 6.0×10^5 and 3.0×10^5 cells per membrane, respectively, on triangular-structured fusion products.

2.7. WST-1 assay

Cell viability was tested with WST-1 (Roche Diagnostics, Mannheim, Germany). Prior to WST-1 testing, fixation rings had to be removed, and the scaffolds, core membranes, and fusion products were relocated to a new 12-well multiwell plate. Cell-populated scaffolds and membranes were incubated with 1:10 diluted WST-1 reagent for 30 minutes at 37 °C. 200 µl of the medium supernatant was then transferred into the cavity of a 96-well plate, and optical density was measured via photometry (Tecan Spektra Rainbow, Tecan, Crailsheim, Germany) at a wave length of 440 nm.

2.8. Bacteria-tightness measurement of the core membranes

Bacteria-tightness was evaluated via an in vitro model. Core membranes were placed into custom-made stainless-steel cell culture inserts and sterilized in ethanol. This way an inside compartment could be separated from an outside compartment (Fig. 4). The inside compartment was then filled with a suspension of ampicillin resistant *E. coli* bacteria in lysogeny broth (LB) medium (in-house production). The outside compartment was filled with pure LB medium. Direct contact between the contaminated inside compartment and non-contaminated outside compartment was only prevented by the core membrane. Bacterial contamination could be detected by the clouding of the LB medium. Turbidity measurement was performed by photometry at a wave length of 595 nm. Control groups consisting of cell culture inserts together with PCL core membranes were also measured with bacterial contamination (inside and outside compartment; positive control) and without bacterial contamination (neither inside nor outside compartment; negative control). Furthermore, the two commercially available collagen-based membranes of Bio-Gide® (Geistlich, Baden-Baden, Germany) and BEGO Collagen Membrane (BEGO, Bremen, Germany) were evaluated in terms of their bacteria-tightness in the same manner.

2.9. Statistical analysis

A statistical analysis was performed using Microsoft Excel 2013 (Microsoft, Redmond, WA, USA) and PRISM (GraphPad Software, CA, USA). We used multiple paired t-tests (two-tailed) to evaluate the cell growth behaviour of different cell types on the scaffolds, membranes, and bacteria tightness of the core membranes. A *p*-value of 0.05 or less was considered to demonstrate statistical significance.

3. Results

3.1. Box-structure scaffolds

All examined scaffolds exhibited continuous cell growth on their surface over the course of 28 days. MG63 cells seeded on box-structure scaffolds with 250 µm fiber spacings showed a 16% higher viability on day 28 than on day 7. This value remained 29%

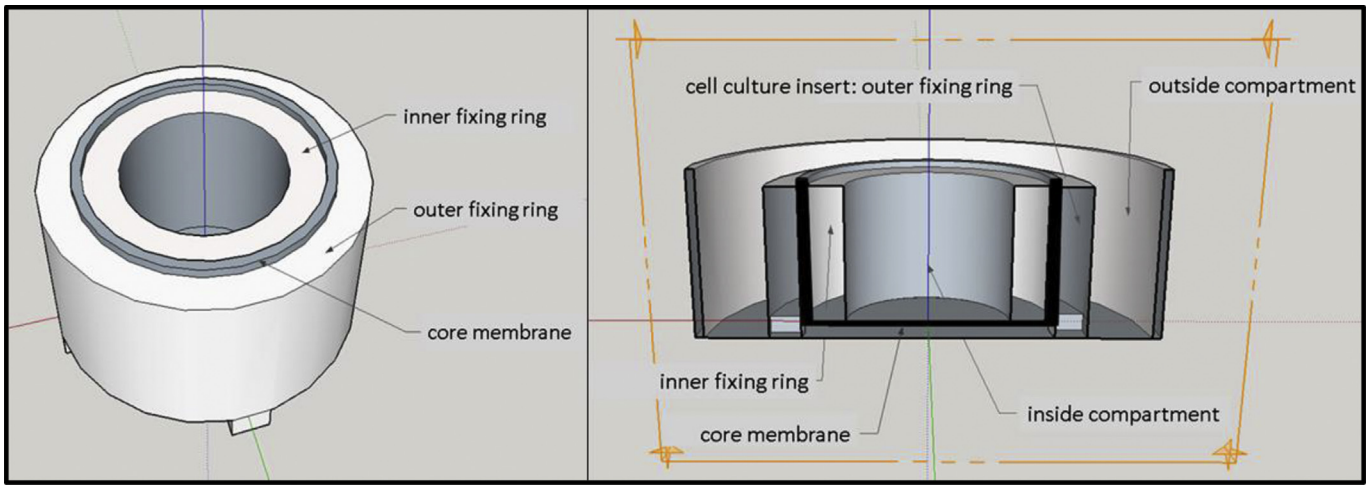

Fig. 4. Schematic of bacterial transmigration experiments through core membranes.

behind the positive control group (Fig. 5a). A similar result was observed for MG63 cells settled on box scaffolds with 500 μm fiber spacings. Viability increased 26% until day 28 and was 48% below the positive control (Fig. 5b). HaCaT cells that were settled on triangular scaffolds with fiber spacings of 250 μm also exhibited steady cell growth after 28 days, beginning at a low-level viability and increasing 136% yet remaining 65% behind the positive control (Fig. 5c). A viability increase of 20% could be seen for L929 cells on the same triangular scaffolds until day 28 of the experiment. This value remained 28% behind the positive control group (Fig. 5d).

3.2. Core

3.2.1. Cytocompatibility

During the course of 4 days, the viability of MG63 cells increased continuously up to a 25% higher viability. Compared with positive controls consisting of cells settled on cell culture dishes (polystyrene) without a membrane, the viability of MG63 cells was significantly lower at all stages (Fig. 6a). Only slightly different results were obtained with HaCaT cells with generally slower growth on core membranes. Viability increased from day 2–3 and decreased until day 4 to a level of 5% below the initial value. Compared with the positive controls, the viability of HaCaT cells was also significantly lower at all stages (Fig. 6b).

3.2.2. Bacteria-tightness of core membranes

On day 1, 94% of the core membranes (30/32) were able to prevent bacterial contamination. 84% of the tested core membranes (28/32) displayed complete bacteria-tightness over 14 days. While continuous bacterial growth occurred in positive control groups, it did not appear in the outside compartment of the core membrane group (Fig. 7a). Extinction values based on bacterial contamination were in the outside compartment, which was protected by PCL membrane cores, significantly lower than in the contaminated inside compartment. At the same time, the extinction values did not differ from the negative control (LB medium). In contrast, none of the collagen-based membrane systems showed full bacteria tightness over the course of 14 days (Fig. 7b, c). Continuous bacterial growth could be observed in the positive control groups as well as in the collagen groups, albeit at a lower level. The measured extinction values were significantly higher for all examined collagen membranes compared with the negative control group (membrane-loaded cell culture inserts in pure LB medium), for which no bacterial growth was found.

3.3. Bilayered core/surface fusion products

Cytocompatibility towards all tested osteoblast-, keratinocyte-, and fibroblast cell lines on bilayered core/surface membranes was slightly enhanced in comparison with core membranes or respective scaffolds alone. After 4 days, osteoblast-like MG63 cells displayed 10% higher viability on 250 μm bilayers than on single core membranes and 15% higher viability than on single 250 μm surface scaffolds (Fig. 8a). Similar results were seen for MG63 cells on 500 μm bilayers, which had higher viabilities of 22% compared with single core membranes and 60% compared with single 500 μm surface scaffolds (Fig. 8b). HaCaT cells on triangular 250 μm

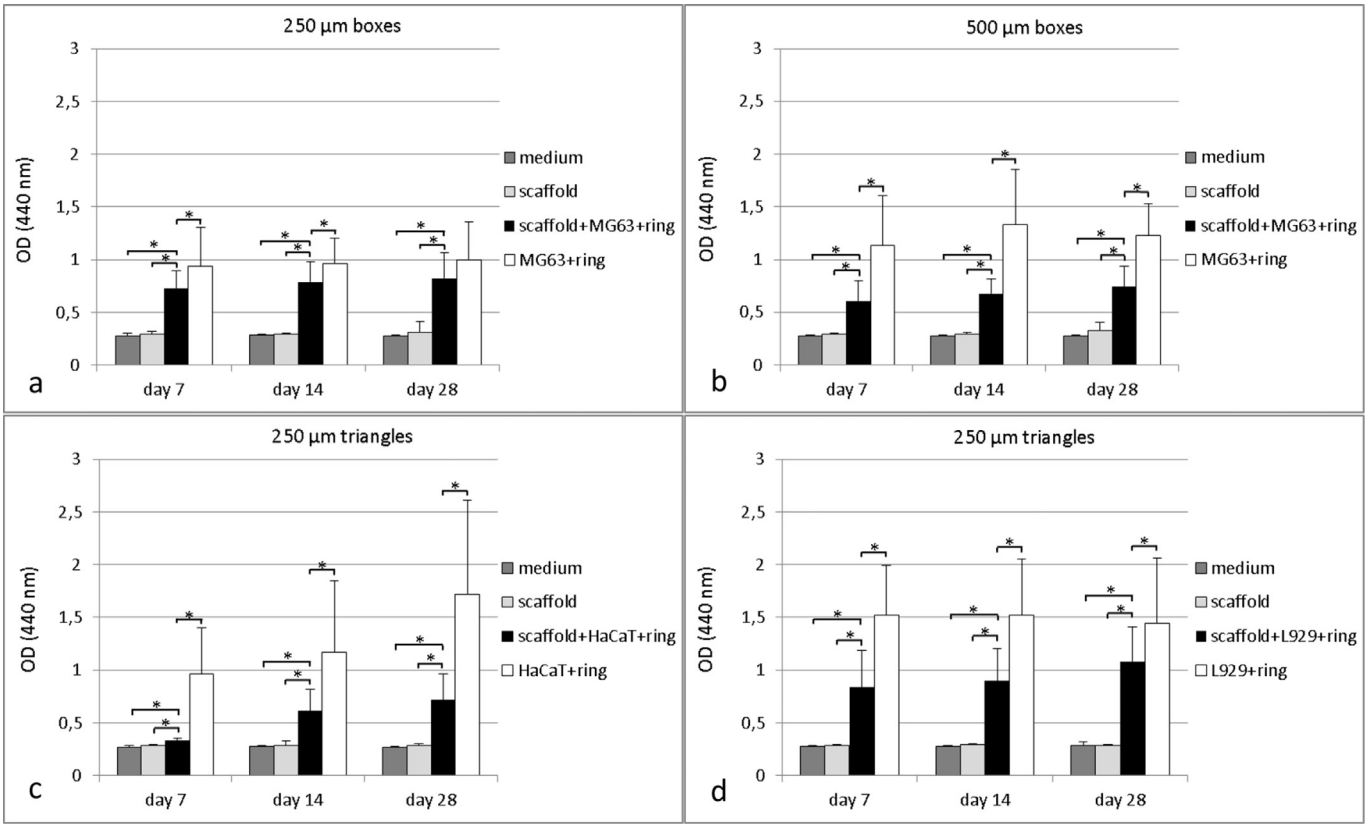

Fig. 5. Cell growth over 28 days on geometric differently arranged scaffolds. Viability of a) MG63 cells on box scaffolds with 250 μm fiber spacings (n = 29), b) MG63 cells on box scaffolds with 500 μm fiber spacings (n = 26), c) HaCaT cells on triangular scaffolds with 250 μm fiber spacings (n = 24), and d) L929 cells on triangular scaffolds with 250 μm fiber spacings (n = 24) according to WST-1 testing after 7, 14 and 28 days of cell culture (asterisks indicate significant differences). **Medium**: pure DMEM with WST reagent, **scaffold**: scaffold burdened with glass ring/beads, **scaffold + MG63/HaCaT/L929**: MG63/HaCaT/L929 cells settled upon respective scaffold burdened with glass ring/beads, **MG63/HaCaT/L929 + ring**: MG63/HaCaTL929 cells settled upon polystyrene cavities of multiwell plates in the presence of glass ring/beads.

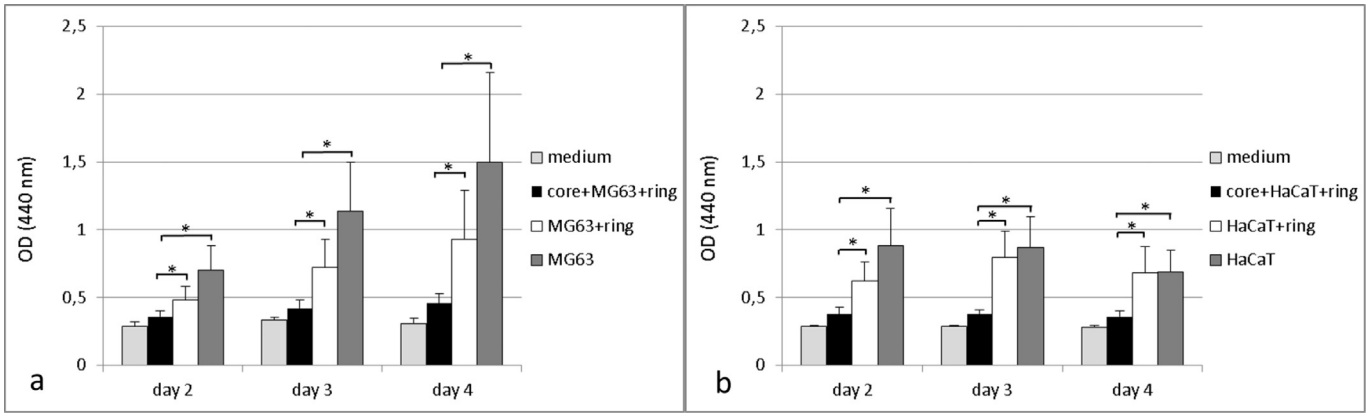

Fig. 6. Cell growth on core membranes. Viability of a) MG63 cells and b) HaCaT cells seeded upon PCL core membranes according to WST-1 testing after 2, 3 and 4 days of cell culture (asterisks indicate significant differences). **Medium**: PCL core membranes burdened with glass ring, **core** + **MG63/HaCaT** + **ring**: MG63/HaCaT cells settled upon PCL core membranes burdened with glass ring, **MG63/HaCaT** + **ring**: MG63/HaCaT cells settled upon polystyrene cavities of multiwell plates in presence of glass ring, **MG63/HaCaT**: Mere cells settled upon polystyrene cavities of multiwell plates.

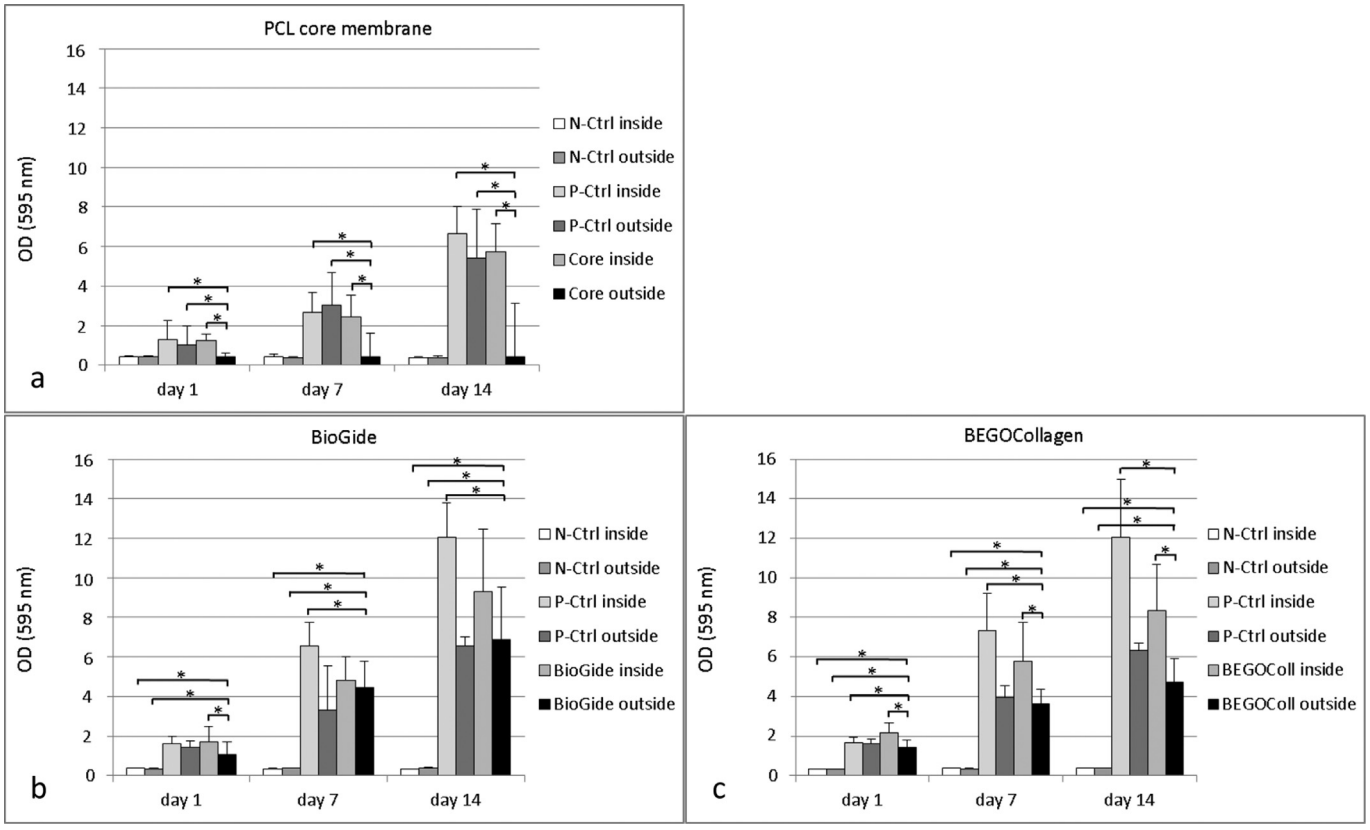

Fig. 7. Transmigration of bacteria through different membranes. Bacterial growth in respective inside- and outside compartments separated by a) PCL core membranes and different collagen-based membranes of b) Bio-Gide® and c) BEGOCollagen (asterisks indicate significant differences). **N-Ctrl**: Membrane-loaded cell culture inserts without E.coli. **P-Ctrl**: Membrane-loaded cell culture inserts loaded with E.coli.

scaffolds also exhibited the same tendency with an increased viability of only 1% on bilayers compared with core membranes and 80% compared with single 250 μm surface scaffolds (Fig. 8c). L929 cells on triangular 250 μm scaffolds displayed increased viability of 16% on bilayers compared to core membranes and 21 % compared with single 250 μm surface scaffolds (Fig. 8d). Viability in comparison with positive controls was always reduced for all types of cells and bilayers.

4. Discussion

The use of membrane systems is relatively wide-spread in oral- and maxillofacial surgery. Membranes are usually placed between bone and oral mucosa and are therefore an interface between these two tissues (Vignoletti et al., 2014). Proper cell growth of respective tissues in the presence of - or rather on top of - membranes is thus of great importance. Using MEW offers the opportunity to fabricate

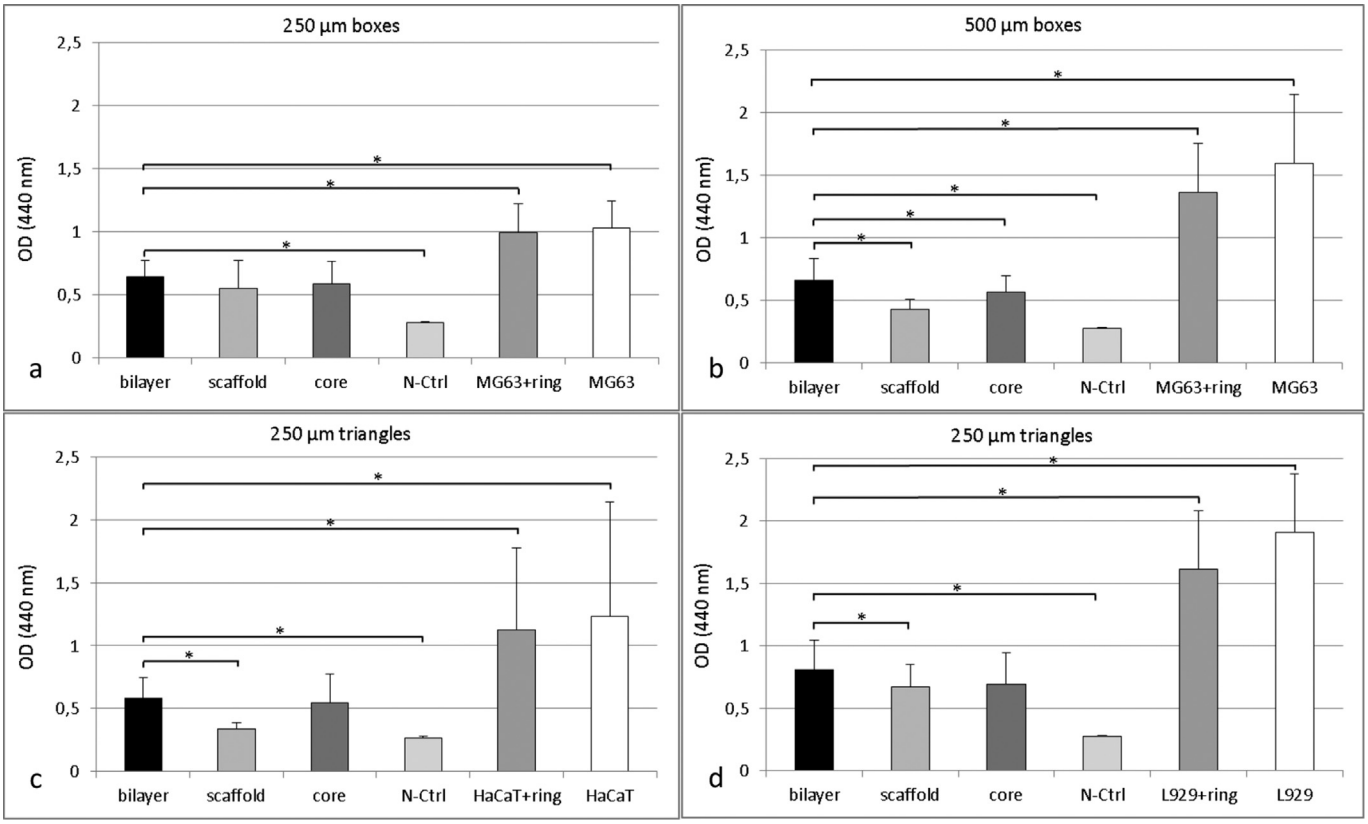

Fig. 8. Cell growth on bilayered membranes compared with single components. Viability of a) MG63 cells seeded on 250 µm box surface scaffolds, b) MG63 cells seeded upon 500 µm box surface scaffolds, c) HaCaT cells seeded on 250 µm triangular surface scaffolds and d) L929 cells seeded on 250 µm triangular surface scaffolds according to WST-1 testing after 4 days of cell culture (asterisks indicate significant differences). **Bilayer**: core membrane fused with surface scaffold, **scaffold**: mere surface scaffold, **core**: mere core membrane, **N-Ctrl**: bilayer burdened with glass ring, **MG63/HaCaT/L929 + ring**: MG63/HaCaT/L929 cells seeded upon polystyrene cavities of multiwell plates in presence of glass ring, **MG63/HaCaT/L929**: Mere cells seeded upon polystyrene cavities of multiwell plates.

scaffolds that allow for optimized cell growth of different types of tissues by varying parameters such as fiber diameters, fiber spacings, surface geometry, and basic material with great preciseness and reproducibility (Youssef et al., 2017; Ruijter et al., 2017; Hrynevich et al., 2018). MEW allows scaffolds to be planned individually and computer-aided before fabrication due to the requirements of different tissue types (Muerza-Cascante et al., 2017). The need for toxic solvents as is often employed within solution electrospinning is obviated, and scaffolds can be processed from medical-grade PCL as received.

Most of the research on electrospun membrane systems thus far has focused on solution-electrospinning, including all its drawbacks (Moheman et al., 2016; Khorshidi et al., 2016). Only a few studies on the use of MEW for manufacturing membranes and scaffolds have been published thus far (Brown et al., 2014, Costa et al., 2014; Chen et al., 2016; Muerza-Cascante et al., 2017; Baldwin et al., 2017), and even fewer have been published on oral- and maxillofacial applications (Haigh et al., 2016; Farag et al. 2018a, 2018b). In this study, we designed and fabricated different types of scaffolds via MEW to enhance the cell growth of oral bone and soft tissues. In so doing, osteoblast-like cells as well as keratinocytes and fibroblasts were seeded on specific scaffolds with differently individualized geometries and fiber spacings that had been chosen on the basis of preliminary studies and literature research which indicated good growth conditions for bone regeneration scaffolds with pore sizes in a range between 95 and 500 µm (Murphy et al., 2010; Perez and Mestres, 2016). Two different surface scaffold designs were chosen because a pursued multilayered membrane is supposed to only be in contact with bone on one side and with epithelium and connective tissue on the other side. Results revealed good cytocompatibility for the respective scaffolds due to cell viability. The cell growth of each cell line increased steadily over 28 days from 16% (MG63 on 250 µm box-shaped scaffolds) to 136% (HaCaT on 250 µm triangular-shaped scaffolds). This finding indicates that scaffolds may be a good growth substrate for cells involved in oral wound healing.

However, such scaffolds alone would be unsuitable for an application in GBR. Due to their relatively large fiber spacings, the basic requirement of cell occlusivity cannot be fulfilled (Dimitriou et al., 2012). Furthermore, one aspired property of our examined membrane was bacteria tightness, which refers to a physical barrier for microorganisms to transmigrate through the membrane. As this could not be performed by scaffolds with large fiber spacings, we produced a bacteria-tight core membrane out of medical-grade PCL via film casting. Current research efforts often have the goal of preventing bacterial infections with membranes. This goal is normally achieved via a processing of antibiotics or anti-infectives on membrane surfaces or inside the membrane structure itself (Owen et al., 2010; Xue et al., 2014, 2015). Using a bacteria-tight membrane may reduce the risk of infection significantly without the use of antibiotics. Again, the initial cell settlement of bone and epithelial cells was assessed for core membranes and revealed a distinct and overall poorer result compared with MEW scaffolds. While osteoblasts showed a relatively good increase in cell viability of 25%, the viability of keratinocytes decreased by 5% through the experiment. However, core membranes displayed excellent properties in terms of their actual goal, namely the prevention of bacterial transmigration. 84% of the examined core membranes showed complete

bacteria tightness over 14 days. The failure rate can most likely be attributed to mechanical damage during the insertion procedure into cell culture inserts, where relatively great shear force was applied to the membranes. However, such strong forces are never applied in regular GBR. In contrast to the PCL core membranes, none of the collagen membranes that are currently most commonly used in daily clinical practice displayed any bacteria tightness.

To compensate for the disadvantages of core membranes regarding decreased cell growth, surface scaffolds were fused with core membranes via heat sealing to form bilayers. An application of multilayered membranes (especially in GBR) has been examined before (Ku et al., 2009; Bottino et al., 2011; Costa et al., 2014; Jeon et al., 2014; Qasim et al., 2017), but never for MEW membranes in combination with core membranes. Attempts with direct MEW of polymer fibers on core membranes failed due to thermic stress. The core membranes constantly melted because of their proximity to the heated nozzle. The cell viability on the bilayers varied according to cell type and surface geometry; nevertheless, bilayers always displayed increased viability compared with core membranes or surface scaffolds alone. The fact that the viability only slightly increased can be partly attributed to the heat-sealing process, in which single components were fused. In this process, three-dimensional structures of scaffolds were compromised in terms of a loss of interconnectivity between polymer fibers as pressure was applied on the pre-warmed scaffolds, and especially the lower fiber layers might have been compressed. However, the surface of the scaffold still preserved the resemblance to the original scaffold, as the assembled construct was rapidly cooled afterwards. The bilayers showed roughly the same viability as the membrane components alone and thus fulfilled their bifunctional task as a bacterial barrier on the one hand and a cell growth promoting substrate on the other hand.

Overall, a multilayered membrane consisting of two surface layers that are connected by one bacteria-tight core membrane should be achieved in the future. A layer-by-layer fabrication via MEW appears to be a good option for reaching this goal, but it is currently not possible due to technical obstacles that remain to be overcome. Moreover, additional investigations on the optimization of surface scaffolds to improve cell adherence and the growth of different tissues have to be conducted. In conclusion, MEW nevertheless represents a new and relatively feasible approach to tissue engineering of membranes for oral and maxillofacial applications. Combining a bacteria-tight core layer with individualized surface layers may enhance the regeneration of oral hard and soft tissues while reducing the risk of undesirable wound infections. This study has thus laid a foundation for this approach to tissue engineering.

5. Conclusion

Membranes with a stratified structure that enhances the growth of tissues involved in oral hard- and soft-tissue regeneration are of great interest in oral and maxillofacial surgery. By using melt electrospinning writing, we fabricated well-defined surface layers (scaffolds) with different geometries out of medical-grade PCL for these membranes that are individualized for the cell attachment of osteoblasts on one side and for keratinocytes and connective tissues on the other side. Furthermore, via film casting, we were able to fabricate a PCL core membrane that links both surface layers. Bacteria-tightness was proven for these core membranes, which may represent a vital contribution towards infection prevention in GTR. By fusing core membranes and surface scaffolds (bilayers), the cell growth of osteoblasts and keratinocytes was even improved compared with individual components.

Funding

This publication was funded by the German Research Foundation (DFG) and the University of Wuerzburg in the funding programme Open Access Publishing.

Additionally, this work was supported by the Interdisciplinary Center for Clinical Research (IZKF) grant Z-3/54.

Conflicts of interest

All authors declare no conflict of interests.

Acknowledgements

We wish to thank Geistlich Biomaterials as well as BEGO for their free supply of membrane samples, which served as comparative groups in the bacteria-tightness testing. Moreover, we want to thank Prof. Paul Dalton for providing advice on MEW. Furthermore, the authors express their appreciation of the personal commitment and help provided by Irina Kucerov in laboratory testing.

References

Arai N, Tsuno H, Okabe M, Yoshida T, Koike C, Noguchi M, et al: Clinical application of a hyperdry amniotic membrane on surgical defects of the oral mucosa. J Oral Maxillofac Surg 70(9): 2221–2228, 2012

Baldwin JG, Wagner F, Martine LC, Holzapfel BM, Theodoropoulos C, Bas O, et al: Periosteum tissue engineering in an orthotopic in vivo platform. Biomaterials 121: 193–204, 2017

Becker ST, Terheyden H, Fabel M, Kandzia C, Möller B, Wiltfang J: Comparison of collagen membranes and polydioxanone for reconstruction of the orbital floor after fractures. J Craniofac Surg 21(4): 1066–1068, 2010

Behring J, Junker R, Walboomers XF, Chessnut B, Jansen JA: Toward guided tissue and bone regeneration: morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes. A systematic review. Odontology 96(1): 1–11, 2008

Birkenfeld F, Behrens E, Kern M, Gassling V, Wiltfang J: Mechanical properties of collagen membranes: are they sufficient for orbital floor reconstructions? J Craniomaxillofac Surg 43(2): 260–263, 2015

Bottino MC, Thomas V, Janowski GM: A novel spatially designed and functionally graded electrospun membrane for periodontal regeneration. Acta Biomater 7(1): 216–224, 2011

Brown TD, Edin F, Detta N, Skelton AD, Hutmacher DW, Dalton PD: Melt electrospinning of poly(ε-caprolactone) scaffolds: phenomenological observations associated with collection and direct writing. Mater Sci Eng C Mater Biol Appl 45: 698–708, 2014

Brown TD, Dalton PD, Hutmacher DW: Melt electrospinning today: an opportune time for an emerging polymer process. Prog Polym Sci 56: 116–166, 2016

Chen F, Hochleitner G, Woodfield T, Groll J, Dalton PD, Amsden BG: Additive manufacturing of a photo-cross-linkable polymer via direct melt electrospinning writing for producing high strength structures. Biomacromolecules 17(1): 208–214, 2016

Costa PF, Vaquette C, Zhang Q, Reis RL, Ivanovski S, Hutmacher DW: Advanced tissue engineering scaffold design for regeneration of the complex hierarchical periodontal structure. J Clin Periodontol 41(3): 283–294, 2014

Dalton PD, Vaquette C, Farrugia BL, Dargaville TR, Brown TD, Hutmacher DW: Electrospinning and additive manufacturing: converging technologies. Biomater 1(2): 171–185, 2013

Dalton PD, Lleixà Calvet J, Mourran A, Klee D, Möller M: Melt electrospinning of poly-(ethylene glycol-block-epsilon-caprolactone). Biotechnol J 1(9): 998–1006, 2006

de Ruijter M, Hrynevich A, Haigh JN, Hochleitner G, Castilho M, Groll J, et al: Out-of-Plane 3D-printed microfibers improve the shear properties of hydrogel composites. Small 14(8). https://doi.org/10.1002/smll.201702773, 2018

Dimitriou R, Mataliotakis GI, Calori GM, Giannoudis PV: The role of barrier membranes for guided bone regeneration and restoration of large bone defects: current experimental and clinical evidence. BMC Med 10(81), 2012

Erdogan Ö, Uçar Y, Tatlı U, Sert M, Benlidayı ME, Evlice B: A clinical prospective study on alveolar bone augmentation and dental implant success in patients with type 2 diabetes. Clin Oral Implant Res 26(11): 1267–1275, 2015

Farag A, Hashimi SM, Vaquette C, Volpato FZ, Hutmacher DW, Ivanovski S: Assessment of static and perfusion methods for decellularization of PCL membrane-supported periodontal ligament cell sheet constructs. Arch Oral Biol 88: 67–76, 2018a

Farag A, Hashimi SM, Vaquette C, Bartold PM, Hutmacher DW, Ivanovski S: The effect of decellularized tissue engineered constructs on periodontal regeneration. J Clin Periodontol 45(5): 586–596, 2018b

Haigh JN, Chuang YM, Farrugia B, Hoogenboom R, Dalton PD, Dargaville TR: Hierarchically structured porous poly(2-oxazoline) hydrogels. Macromol Rapid Commun 37(1): 93–99, 2016

Hochleitner G, Jüngst T, Brown TD, Hahn K, Moseke C, Jakob F, et al: Additive manufacturing of scaffolds with sub-micron filaments via melt electrospinning writing. Biofabrication 7(3): 035002, 2015
Hrynevich A, Elçi BŞ, Haigh JN, McMaster R, Youssef A, Blum C, et al: Dimension-based design of melt electrowritten scaffolds. Small 14(22): e1800232, 2018
Hutmacher DW, Dalton PD: melt electrospinning. Chem Asian J 6(1): 44–56, 2011
Jegoux F, Malard O, Goyenvalle E, Aguado E, Daculsi G: Radiation effects on bone healing and reconstruction: interpretation of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 109(2): 173–184, 2010
Jeon JE, Vaquette C, Theodoropoulos C, Klein TJ, Hutmacher DW: Multiphasic construct studied in an ectopic osteochondral defect model. J R Soc Interface 11(95): 20140184, 2014
Jonker BP, Roeloffs MW, Wolvius EB, Pijpe J: The clinical value of membranes in bone augmentation procedures in oral implantology: A systematic review of randomised controlled trials. Eur J Oral Implantol 9(4): 335–365, 2016
Khorshidi S, Solouk A, Mirzadeh H, Mazinani S, Lagaron JM, Sharifi S, et al: A review of key challenges of electrospun scaffolds for tissue-engineering applications. J Tissue Eng Regen Med 10(9): 715–738, 2016
Ku Y, Shim IK, Lee JY, Park YJ, Rhee SH, Nam SH, et al: Chitosan/poly(L-lactic acid) multilayered membrane for guided tissue regeneration. J Biomed Mater Res A 90(3): 766–772, 2009
Lian H, Meng Z: Melt electrospinning vs. solution electrospinning: a comparative study of drug-loaded poly (ε-caprolactone) fibres. Mater Sci Eng C Mater Biol Appl 74: 117–123, 2017
Mardas N, Schwarz F, Petrie A, Hakimi AR, Donos N: The effect of SLActive surface in guided bone formation in osteoporotic-like conditions. Clin Oral Implant Res 22(4): 406–415, 2011
Moheman A, Alam MS, Mohammad A: Recent trends in electrospinning of polymer nanofibers and their applications in ultra thin layer chromatography. Adv Colloid Interface Sci 229: 1–24, 2016
Muerza-Cascante ML, Shokoohmand A, Khosrotehrani K, Haylock D, Dalton PD, Hutmacher DW, et al: Endosteal-like extracellular matrix expression on melt electrospun written scaffolds. Acta Biomater 52: 145–158, 2017
Murphy CM, Haugh MG, O'Brien FJ: The effect of mean pore size on cell attachment, proliferation and migration in collagen-glycosaminoglycan scaffolds for bone tissue engineering. Biomaterials 31(3): 461–466, 2010
Owen GR, Jackson JK, Chehroudi B, Brunette DM, Burt HM: An in vitro study of plasticized poly(lactic-co-glycolic acid) films as possible guided tissue regeneration membranes: material properties and drug release kinetics. J Biomed Mater Res A 95(3): 857–869, 2010
Perez RA, Mestres G: Role of pore size and morphology in musculo-skeletal tissue regeneration. Mater Sci Eng C Mater Biol Appl 61: 922–939, 2016
Qasim SB, Najeeb S, Delaine-Smith RM, Rawlinson A, Ur Rehman I: Potential of electrospun chitosan fibers as a surface layer in functionally graded GTR membrane for periodontal regeneration. Dent Mater 33(1): 71–83, 2017
Qin CC, Duan XP, Wang L, Zhang LH, Yu M, Dong RH, et al: Melt electrospinning of poly(lactic acid) and polycaprolactone microfibers by using a hand-operated Wimshurst generator. Nanoscale 7(40): 16611–16615, 2015
Rinna C, Ungari C, Saltarel A, Cassoni A, Reale G: Orbital floor restoration. J Craniofac Surg 16(6): 968–972, 2005
Sampath M, Lakra R, Korrapati P, Sengottuvelan B: Curcumin loaded poly (lactic-co-glycolic) acid nanofiber for the treatment of carcinoma. Colloids Surf B Biointerfaces 117: 128–134, 2014
Santocildes-Romero ME, Hadley L, Clitherow KH, Hansen J, Murdoch C, Colley HE, et al: Fabrication of electrospun mucoadhesive membranes for therapeutic applications in oral medicine. ACS Appl Mater Interfaces 9(13): 11557–11567, 2017
Scheller EL, Krebsbach PH, Kohn DH: Tissue engineering: state of the art in oral rehabilitation. J Oral Rehabil 36(5): 368–389, 2009
Schwarz L, Schiebel V, Hof M, Ulm C, Watzek G, Pommer B: Risk factors of membrane perforation and postoperative complications in sinus floor elevation surgery: review of 407 augmentation procedures. J Oral Maxillofac Surg 73(7): 1275–1282, 2015
Vignoletti F, Matesanz P, Rodrigo D, Figuero E, Martin C, Sanz M: Surgical protocols for ridge preservation after tooth extraction. A systematic review. Clin Oral Implant Res 23(Suppl. 5): 22–38, 2012
Vignoletti F, Nunez J, Sanz M: Soft tissue wound healing at teeth, dental implants and the edentulous ridge when using barrier membranes, growth and differentiation factors and soft tissue substitutes. J Clin Periodontol 41(Suppl. 15): 23–35, 2014
Xue J, Shi R, Niu Y, Gong M, Coates P, Crawford A, et al: Fabrication of drug-loaded anti-infective guided tissue regeneration membrane with adjustable biodegradation property. Colloids Surf B Biointerfaces 135: 846–854, 2015
Xue J, He M, Niu Y, Liu H, Crawford A, Coates P, et al: Preparation and in vivo efficient anti-infection property of GTR/GBR implant made by metronidazole loaded electrospun polycaprolactone nanofiber membrane. Int J Pharm 475(1–2): 566–577, 2014
Yonezawa H, Yanamoto S, Kawasaki G, Umeda M: Polyglycolic acid sheets with fibrin glue (MCFP technique) for resection of oral mucosa. Br J Oral Maxillofac Surg 50(3): 43–44, 2012
Youssef A, Hollister SJ, Dalton PD: additive manufacturing of polymer melts for implantable medical devices and scaffolds. Biofabrication 9(1): 012002, 2017
Zhang J, Xu Q, Huang C, Mo A, Li J, Zuo Y: Biological properties of an anti-bacterial membrane for guided bone regeneration: an experimental study in rats. Clin Oral Implant Res 21(3): 321–327, 2010